GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

PROJECT ADMINISTRATION DATA SHEET

[X] ORIGINAL

[ ] REVISION NO. ____

Project No. G-33-K04 DATE: 4/3/81

Project Director: C.L. Hall School/~~Dept~~ Chemistry

Sponsor: DHEW/PHS/NIH - Nat'l Institute of General Medical Sciences; Bethesda, MD. 20014

Type Agreement: Grant No. 2 R01 GM25494-04

Award Period: From 7/1/81 To 6/30/82 (Performance) 9/30/82 (Reports)

Sponsor Amount: $107,737 PHS funds G-33-K04 Contracted through: ~~GTRC~~/GIT

Cost Sharing: $5,670 GIT G-33-359

Title: Studies on Fatty ACYL COA Dehydrogenase and ETF

ADMINISTRATIVE DATA OCA CONTACT Don Hasty

1) Sponsor Technical Contact: Dr. David Beck, Program Administrator, National Institute of General Medical Sciences, Bethesda, MD. 20014 - Phone (301)496-7175

2) Sponsor Admin./Contractual Contact: Ms. Ruth Monaghan/Linda Glen, Grants Management Specialists, Office of Associate Director for Program Activities, National Institute of General Medical Sciences, Bethesda, MD. 20014 - Phone (301)496-7746

Reports: See Deliverable Schedule Security Classification: none

Defense Priority Rating: n/a

RESTRICTIONS

See Attached NIH Supplemental Information Sheet for Additional Requirements.

Travel: Foreign travel must have prior approval - Contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of $500 or 125% of approved proposal budget category.

Equipment: Title vests with GIT

COMMENTS: Follow-on to Project G-33-K03

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SP500 R-2

GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION SHEET

Date 10/18/82

Project Title: Studies on Fatty ACYL COA Dehydrogenase and ETF

Project No: G-33-K04

Project Director: Dr. Carole L. Hall

Sponsor: DHEW/PHS/NIH; National Institute of General Medicine, Bethesda, MD. 20014

Effective Termination Date: 9/30/82 (04 year)

Clearance of Accounting Charges: -----

Grant/Contract Closeout Actions Remaining:

- ☐ Final Invoice and Closing Documents
- ☐ Final Fiscal Report
- ☐ Final Report of Inventions
- ☐ Govt. Property Inventory & Related Certificate
- ☐ Classified Material Certificate
- ☒ Other Annual Report of Expenditures (04 year)

NOTE: Followed by G-33-K05

Assigned to: Chemistry (School/~~Laboratory~~)

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FORM OCA 10:781

G-33-F04

SECTION IV

APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE 1	GRANT NUMBER
SECTION IV—SUMMARY PROGRESS REPORT	1 R01 GM 25494-05
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial) Hall, Carole L.	PERIOD COVERED BY THIS REPORT: FROM July 1,1981- THROUGH June 30,1982
NAME OF ORGANIZATION School of Chemistry, Georgia Tech.	

TITLE (Repeat title shown in Item 1 on first page)

Fatty acyl CoA dehydrogenases and Electron Transfer Flavoprotein

1. List all publications, not previously reported, resulting from work supported by this grant (author(s), title, page numbers, year, journal or book). List manuscripts separately as submitted for publication or accepted for publication.
2. Provide two reprints of publications not previously submitted to the awarding unit.
3. Progress Report. (See instructions)

1.a. Hall, Carole L. "Acyl CoA Dehydrogenases from Pig liver Mitochondria" in J.M. Lowenstein (Ed.) Methods in Enzymology (1981) 71,375-385.

b. Hall, Carole L. "Electron-Transfer Flavoprotein from Pig Liver Mitochondria" Ibid.,pp 386-390.

c. Bell, J.E. and Hall, C.L. "Ultraviolet and Visible Absorbance Spectroscopy" in J.E.Bell (Ed.)Spectroscopy in Biochemistry, Vol. I, (1981) CRC Press, Boca Raton Fla. pp3-62.

d. Rhead, W.J. , Hall, C.L. and Tanaka, K. "Novel Tritium Release Assays for Isovaleryl-CoA and Butyryl-CoA Dehydrogenases (1981) J. Biol. Chem. 256, 1616-1624.

e. Hall, C.L. and Lambeth, J.D. "Electron Transfer from Acyl CoA to General Acyl CoA Dehydrogenase and ETF" (1981) in Flavins and Flavoproteins, K. Yagi and T. Yamano, (Eds) Japan Scientific Press, Tokyo, pp 657-667.

f. Hall, C.L. "Isovaleryl CoA-Dehydrogenase (IV-D) from Pig Liver Mitochondria" (1981) Walser and Williamson,(Eds) Metabolism and Clinical Implications of Branched Chain Amino and Ketoacids. Elsevier North Holland, Inc. pp 35-40.

g. Hall, C.L. "On the Dehydrogenation of Octanoyl CoA by General Acyl CoA Dehydrogenase and ETF." (1982) Flavins and Flavoproteins, V.Massey and C.H. Williams, Jr. (Eds.) Elsevier North Holland,(Published but not yet available).

h. Hall, C.L. "Effects of Concentration and Temperature on Reduction of Electron Transfer Flavoprotein by General Acyl CoA Dehydrogenase and Octanoyl CoA." (1982) Fed. Proc.

2. See Appendix. (numbered as under #1 above).

3. Progress Report

A. The general objectives and specific aims of the project are the same as those proposed in the last competitive review (October, 1980).

B. I.Additional work on characterizing the isovaleryl CoA dehydrogenase(IV-D) has been carried out. A preliminary report has been published (Hall, C.L., in Metabolism and Clinical Implications of Branched Chain Amino and Ketoacids, see above and Appendix). Preliminary studies on the interaction of IV-D with ETF have been done, but studies in the stopped flow spectrophotometer have been hampered by difficulties in obtaining IV-D samples of sufficient purity in sufficient quantity. Recent development of more sensitive fluorescence techniques (see v. below) should permit assessment of interaction parameters using much smaller amounts of IV-D than ETF. A more detailed manuscript on the characterization of the enzyme is in preparation.

Progress Report (Cont'd)

3. B. II. Attempts have been made to show the release of octenoyl CoA concomitant with ETF reduction. These studies have been difficult and tedious, using rather large amounts of enzymes, but show that stoichiometrec conversion of saturated acyl CoA to unsaturated acyl CoA does not occur, and that amounts of octenoic-methyl ester approximately equal to ETF flavin content can be found free in solution only after reoxidation of ETF. Some octenoyl CoA appears also to be bound to ETF, as it could only be removed by extraction of denatured enzyme. Some of these results were presented at the 7th International Symposium on Flavins and Flavoproteins, which has been recently published, but reprints are not yet available. See abstract in Appendix.

III. Studies on the isolation of a short-medium and a different medium-long chain acyl CoA dehydrogenase and ETF from Pseudomonas oleovorans have been carried out. These enzyme preparations are still contaminated by other proteins but can be shown to have structural and kinetic properties similar to their mammalian counterparts. This study was partially supported by a BSRG grant to C.L.H. June 1980-March 1981.

IV. Studies on the effect of dehydrogenase concentrations and temperature on the reduction of ETF by G-AD and C_8CoA have been carried out on a computer-assisted stopped flow spectrophotometer. The instrument has kindly been made available to me by Dr. D. B. McCormick, Dale Edmondson and J.D. Lambeth of the Department of Biochemistry at Emory University. The use of computer fitting programs to analyze the complicated kinetics of this interaction has greatly facilitated studies on the interaction of dehydrogenase and ETF. The studies showed marked differences in the effects of both G-AD concentration and temperature upon reduction of ETF to the semiquinone as compared to the subsequent reduction to ETF hydroquinone. A preliminary report was presented at the 1982 FASEB meeting in New Orleans, La. April, 1982. (See Appendix for abstract).

V. Studies leading to development of a sensitive assay for acyl CoA dehydrogenases in whole (broken) mitochondrial fractions and crude liver homogenate samples have been carried out using pig liver fractions. These studies were extended to human autopsy liver tissue provided me by Dr. L. Goodman of the University of Colorado Childrens Hospital (originally submitted to me for ETF analysis). Recent studies with collaborators at the University of Pennsylvania (Drs. Barbara Corkey; John Williamson, Jr; Daniel Hale, Jr; Paul Coates and Charles Stanley) have demonstrated the usefulness of these techniques using liver biopsy material in identifying a deficiency in general acyl CoA dehydrogenase but not short chain, long-chain or isovaleryl CoA dehydrogenases. A manuscript describing the assay technique, which depends on the loss of fluorescence of pig liver ETF upon reduction by catalytic amounts of dehydrogenase and the appropriate substrate, is in preparation, as is a manuscript describing the patient studies.

VI. Studies leading to synthesis and purification of α,β dideutero C_8CoA have been carried out since kinetic isotope effects upon the reduction of G-AD and ETF are expected to shed light on the mechanism of the electron transfers involved in the reduction of ETF. Pilot studies have shown that 90% dideutero-octanoic acid can be prepared from a mixture of cis and trans-octenoic acid. The stereochemistry of the reaction is well known to be 90% specific for cis additions, and the yield of saturated(dideutero) octanoic acid (measured as the methyl ester) from the cis,trans mixture is about 60%. In other pilot studies, oxalyl chloride activation of octanoic and of trans-2-octenoic acid have been accomplished, as well as esterification to CoA. Techniques for separating the saturated (dideutero) fatty acid from the remaining trans,2-octenoic acid are being tested.

Progress Report (Cond'd)

3. C. Specific Objectives

I. Different chromatographic techniques to better separate IV-D from G-AD and LC-AD will be explored in the hope of preparing IV-D of suffiecient quantity and purity to carry out stopped flow spectrophotometric studies on the reduction of IV-D by IVCoA and upon the reduction of ETF by IV-D and IVCoA . Studies on the reduction of ETF by IV-D and IVCoA will also be carried out by fluorescence assay techniques using catalytic rather than stoichiometric amounts of dehydrogenase (see 3.B.V. above).

II. Studies on substrate and/or product inhibition effects on the reduction of ETF will be carried out both on the computer-assisted stopped-flow spectrophotometer at Emory(as in 3.B.IV. above) and using the catalytic fluorescence assay(as in 3.B. V above). The latter requires collection and analysis of the pseudo-first order fluorescence-loss progress curves best carried out by computer. Other, even more complicated graphical analysis of pseudofirst order progress curves have been proposed and used. Data collection and analysis of this type requires the use of a dedicated computer for the many experiments which could be devised and which are expected to provide much useful information. Dr. Richard De Sa of On Line Instrumentation Systems has graciously consented to allow me to purchase parts of his excellent hardware and software package (See budget justifications for current grant year and next grant year, sections II and III, this application). Additional funds for hardware and software are being requested from the BSRG committee at Georgia Tech for June 1982-March 1983.

III. Studies on the reduction of ETF by G-AD but using short and long chain acyl CoA substrates, as well as studies on the reduction of ETF by SC-AD by the techniques outlined under 3.B. I and 3.B.V (above) will be carried out and should provide information on the overall mechanism of reduction of ETF by acyl CoA dehydrogenases.

IV. Further studies on product formation ulilizing HPLC equipment available to me in the laboratory of Dr. Leon Zalkow, School of Chemistry, Georgia Tech and techniques developed by Dr. Barbara Corkey and graciously made available to me by her will also be carried out in parallel to the studies outlined above to continue the studies outlined in 3.B.II (above).

V. Synthesis of α,β cis dideuterooctanoyl CoA will be carried out as described under 3.B.VI. (above) and used in stopped-flow spectrophotometric studies on the reduction of G-AD and ETF, similar to studies described in Hall, Lambeth and Kamin (1979) J. Biol. Chem. 254 2033-2043 and in Hall and Lambeth,(1980) J. Biol. Chem. 255, 3541, and in 3.B.IV (above).

VI. Further attempts to purify acyl CoA dehydrogenases and ETF from Pseudomonas oleovorans will be made. The enzymes have been partially purified but are still not homogeneous. When pure, structural and catalytic characterization studies will be made.